RESEARCH HIGHLIGHT

Designing de novo D-protein binders

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D-proteins hold considerable potential in biotechnological applications because of their generally anticipated higher bioorthogonality and stability over L-proteins. In a recent paper in *Cell Research*, Sun and colleagues demonstrated the successful design of de novo D-proteins that selectively bind to specific target regions of natural L-proteins or L-peptides.

Unlike natural 'proteins composed of L-amino acid residues, D-proteins are built of D-amino acid residue units. Being not the substrates of natural proteases or the natural ligands of cellular receptors, D-proteins are generally expected to be of higher stability and bioorthogonality over L-proteins in various applications. Therefore, effective and generalizable techniques that can identify new D-proteins as selective binders of any given L-protein target are highly sought after.[1,2] One approach adapts existing wet-experiment methods to screen for such abiotic binders by performing high-throughput experimental screening in the mirror-image space.[3] Namely, a D-protein target corresponding to the mirror image of the original L-protein target is used as a bait to filter out binders from phage- or cell surface-displayed libraries of L-proteins. The identified binder L-proteins are mirror imaged to yield the desired D-proteins. There are two unmet challenges with such a purely wet-experiment-based approach. First, it is not guaranteed that the initial random libraries indeed contain proper binders. Second, it is very difficult to obtain binders that selectively recognize a specific region of the target protein.

Progresses in computational design of binder proteins have offered an approach with features addressing these two challenges to complement experimental screening.[4–6] Previously, L-proteins that bind to specific surface regions of natural protein targets have been successfully obtained through computational protein design with models mixing physics-based and data-driven components. However, the design of D-proteins to target L-proteins is considered to be much more challenging, because there are only a few available experimentally determined structures of heterochiral D-protein–L-protein complexes. It was unclear to what extent the existing computational protein design models, with their data-driven components developed based on the structures of L-proteins, could be adapted and applied to design and evaluate heterochiral complexes.

Sun and colleagues developed an approach integrating computational protein design and experimental screening to obtain binder D-proteins for given L-protein targets (Fig. 1).[7] As before, the procedures were carried out in the mirror-image space by using chemically synthesized D-protein targets instead of the original natural L-protein targets to facilitate the experimental screening and directed evolution of binder L-proteins. The authors developed their computational protocol by hypothesizing that the heterochiral protein–protein interactions adhere to the same principles of maximizing the shape and chemical complementarity between the interacting surfaces as the interactions between L-proteins. The authors tried to quantify such complementarity with the binding energies and interface metrics computed with the Rosetta program, and found that these quantities calculated for heterchiral protein–protein and protein–peptide complexes available in PDB were in similar ranges as observed for complexes of L-proteins.

The actual design process started with the generation of a so-called rotamer interaction field (RIF) using the program named RifDock,[4] which involved the sampling and ranking of a large number of poses of discrete L-residue rotamers docked at selected surface regions of the D-protein targets. The resulting RIF was used to guide the docking of 9606 pre-designed scaffolds (i.e., backbone structures) of miniproteins against the target. Interface residues were designed on the docked scaffolds. The MotifGraft algorithm[8] was used to sample more backbone geometries and interface residue compositions. The designed miniprotein sequences were experimentally screened for their binding against the chemically synthesized D-protein targets by yeast display Some of the resulting binders were further optimized through directed evolution experiments, in which site saturation mutagenesis, mutation combination and random mutagenesis libraries were integrated.

The authors demonstrated their approach by testing one artificial alpha helical peptide target (L-Pep-1) and two natural L-proteins of pharmacological interest (the D5 domain of human tropomyosin receptor kinase A (L-TrkA) and the human interleukin-6 (L-IL-6)). For the two natural protein targets, the binding regions targeted by the designs corresponded to the binding interfaces of the respective natural binders (nerve growth factor for L-TrkA and the IL-6 receptors for L-IL-6). There were no previously known D-proteins binding these targets. Computational design explored local minima in an estimated sequence space of 10^{20} and generated 4500–14,000 candidate miniprotein binders for each (mirror-imaged) target. These candidates were subjected to subsequent experimental screening by yeast display. The most enriched miniproteins from the screening experiments were individually expressed, purified, and their binding affinities to the corresponding targets were characterized. The measured binding constants were 22–125 nM. Binding assays using mutated target proteins confirmed that the interactions were dependent on the residue types in the targeted surface regions. After further directed evolution, the authors eventually obtained binders with affinities of 7.8 nM for D-TrkA and 0.7 nM for D-IL-6.

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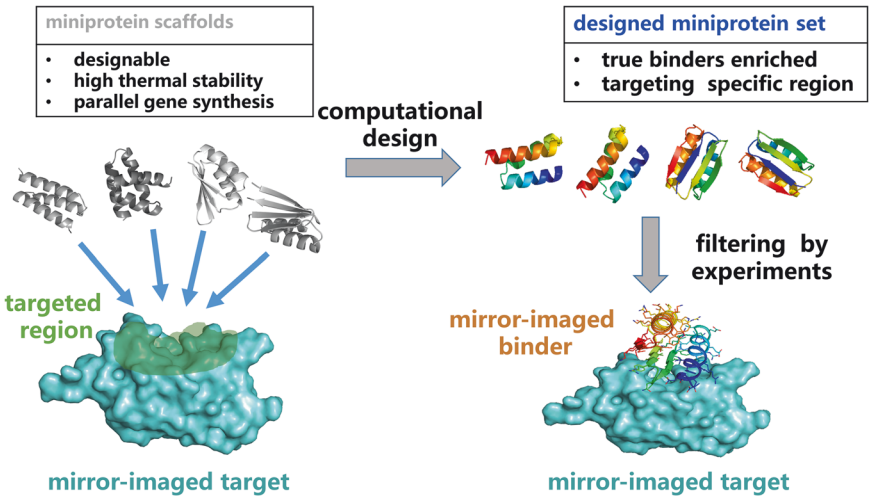

Fig. 1 Design and selection in the mirror-image space. Miniprotein binders were first computationally designed and then experimentally screened by experiments using mirror-imaged D-protein targets. The main advantages of using miniprotein scaffolds and computational design are listed in the boxes.

Finally, the authors synthesized and characterized the D-protein versions of the high-affinity miniprotein binders. They found that the D-proteins indeed bind to their respective L-protein targets with expected affinities. The interactions were shown to be of high target selectivity and enantiomer selectivity. In cell-based experiments, the D-protein binders for L-TrkA and L-IL-6 were shown to compete with the corresponding natural ligand/receptor to block downstream signaling pathways. Additionally, the D-proteins exhibited hyperthermal stability (with melting temperatures above 95 °C as indicated by temperature-dependent circular dichroism spectra) and strong resistance to proteolysis by common proteases.

The authors were able to determine the crystal structure of a designed binder D-protein in complex with L-Pep-1, which was of nearly identical backbone structure to the design model. The structure was also almost identical to the mirror image of the crystal structure determined for the corresponding binder L-protein in complex with D-Pep-1.

Together, the results presented by Sun and colleagues demonstrated that designer D-protein binders of L-proteins can be effectively obtained with an integrated approach of computational design and experimental screening in the mirror-image space. The advantages of this approach include that the binders are of hyperstability and can be generated against specific surface regions of the targets. As highlighted by the authors, the ability to design such binders paved the way for a diverse range of applications.

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ADDITIONAL INFORMATION

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